Potential Mechanisms of Thyroid Disruption in Humans: Interaction of Organochlorine Compounds with Thyroid Receptor, Transthyretin, and Thyroid-binding Globulin

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Organochlorine compounds, particularly polychlorinated biphenyls (PCBs), alter serum thyroid hormone levels in humans. Hydroxylated organochlorines have relatively high affinities for the serum transport protein transthyretin, but the ability of these compounds to interact with the human thyroid receptor is unknown. Using a baculovirus expression system in insect cells (Sf9 cells), we produced recombinant human thyroid receptor β (hTRβ). In competitive binding experiments, the recombinant receptor had the expected relative affinity for thyroid hormones and their analogs. In competitive inhibition experiments with PCBs, hydroxylated PCBs (OH-PCBs), DDT and its metabolites, and several organochlorine herbicides, only the OH-PCBs competed for binding. The affinity of hTRβ for OH-PCBs was 10,000-fold lower (K_i = 20–50 μM) than its affinity for thyroid hormone (3,3′,5-triiodothyronine, T_3; K_i = 10 nM). Because their relative affinity for the receptor was low, we tested the ability of OH-PCBs to interact with the serum transport proteins—transthyretin and thyroid-binding globulin (TBG). With the exception of one compound, the OH-PCBs had the same affinity (K_i = 10–80 nM) for transthyretin as thyroid hormone (thyroxine; T_4). Only two of the OH-PCBs bound TBG (K_i = 3–7 μM), but with a 100-fold lower affinity than T_4. Hydroxylated PCBs have relatively low affinities for the human thyroid receptor *in vitro*, but they have a thyroid hormonelike affinity for the serum transport protein transthyretin. Based on these results, OH-PCBs *in vivo* are more likely to compete for binding to serum transport proteins than for binding to the thyroid receptor. *Key words*: endocrine disruption, PCB, thyroid-binding globulin, thyroid receptor, transthyretin. *Environ Health Perspect* 107:273–278 (1999). [Online 9 March 1999] *http://ehpnet1.niehs.nih.gov/docs/1999/107p273-278cheek/abstract.html*

An important question in endocrine disruption is the mechanism by which a xenobiotic compound alters the action of endogenous hormones. One possible mechanism is direct interaction with the hormone receptor, either as an agonist or as an antagonist. In the case of thyroid hormone, a second important mechanism may be the ability of compounds to alter serum transport of thyroid hormones (TH). In nonmammalian vertebrates, the major transport protein is prealbumin (transthyretin); while some mammals, including humans, have a second binding protein, thyroid-binding globulin (TBG) (*1*). Assessing the relative affinity of the thyroid receptor and the serum transport proteins for xenobiotics should help clarify one of the mechanisms by which xenobiotics alter thyroid homeostasis.

Alterations in thyroid homeostasis by organochlorine compounds have been documented for many species, including humans. In most cases, exposure to organochlorine compounds is correlated with decreased serum levels of thyroid hormone, particularly thyroxine (T_4). Exposure to polychlorinated biphenyls (PCBs) has been correlated with decreased serum T_4 concentrations in rats (*2–10*) and humans (*6,11,12*). Evidence from rat studies indicates that PCB-induced decreases in serum T_4 are the result of increased metabolism by uridine diphosphate glucuronysyltransferase (UDPGT), a hepatic enzyme that glucuronidates T_4 (*5,13–15*). Another class of organochlorines, the choroacetanilides acetochlor and alachlor, elevates UDPGT activity and concomitantly decreases serum T_4 levels in rats (*16,17*). Acetochlor also alters thyroid hormone (3,3′,5-triiodothyronine; T_3) action in amphibians, accelerating T_3-induced metamorphosis (*18*). DDT and its metabolites alter serum T_4 levels in birds (*19*) and humans (*20*). DDT also alters thyroid metabolism in rats by increasing hepatic UDPGT activity (*21*).

Because of their physiological effects and their structural resemblance to thyroid hormones (Fig. 1), several studies have investigated the ability of PCBs to bind to the serum transport proteins transthyretin (*3,8,22–24*) and TBG (*22*) and to the rat thyroid receptor (*23*). Transthyretin (TTR) and TBG have similar affinities for the natural ligand, T_4 (50–90 nM) (*22*), but have different affinities for PCBs. Hydroxylated PCBs are potent ligands for TTR, having affinities in the 1 nM range, 50-fold greater than that of T_4 (*8,22,24*). Few hydroxylated PCBs bind TBG (*22*) and few unmetabolized PCBs have strong affinities for either TTR or TBG (*8,22,23*). Like the transport proteins, the rat thyroid receptor appears to have a higher affinity for hydroxylated versus parent PCBs (*23*).

Although studies of binding affinity suggest that organochlorine compounds may alter thyroid homeostasis by interacting with thyroid hormone transport proteins in humans and animals, little is known about the ability of organochlorines to interact with the thyroid receptor, particularly in humans. We examined the ability of PCBs, DDTs, chloroacetanilides, and an isoprenoid to bind a recombinant human thyroid receptor (hTRβ1). To evaluate the relative significance of receptor versus transport protein binding for disrupting thyroid homeostasis, compounds that bound the receptor were also tested for binding to human transthyretin and TBG.

Methods

Chemicals

T_3, T_4, 3,3′,5-triiodothyroacetic acid (Triac), 3,3′,5,5′-tetraiodothyroacetic acid (Tetrac), 3,3′,5′-triiodo-L-thyronine (rT_3), 2,2-bis(*p*-chlorophenyl)-ethanol (DDOH), human TBG, and human transthyretin (prealbumin) were purchased from Sigma Chemical Co. (St. Louis, MO). The PCBs—3,3′,4,4′,5-pentachlorobiphenyl (PCB 126), 3,3′4,4′-tetrachlorobiphenyl (PCB 77), 3-OH-2′,4′,6′-trichlorobiphenyl, 4-OH-3,5-dichlorobiphenyl, 4-OH-2′,3′,4′,5′-tetrachlorobiphenyl, 4-OH-2′,3,4′,6′-tetrachlorobiphenyl, 4-OH-2′,3,3′,4′,5′-pentachlorobiphenyl, and 4-OH-2′,3,4′,5,6′-pentachlorobiphenyl—were purchased from AccuStandard (New Haven, CT). The hydroxylated PCB, 4,4′-diOH-3,3′,5,5′-tetrachlorobiphenyl, was purchased from

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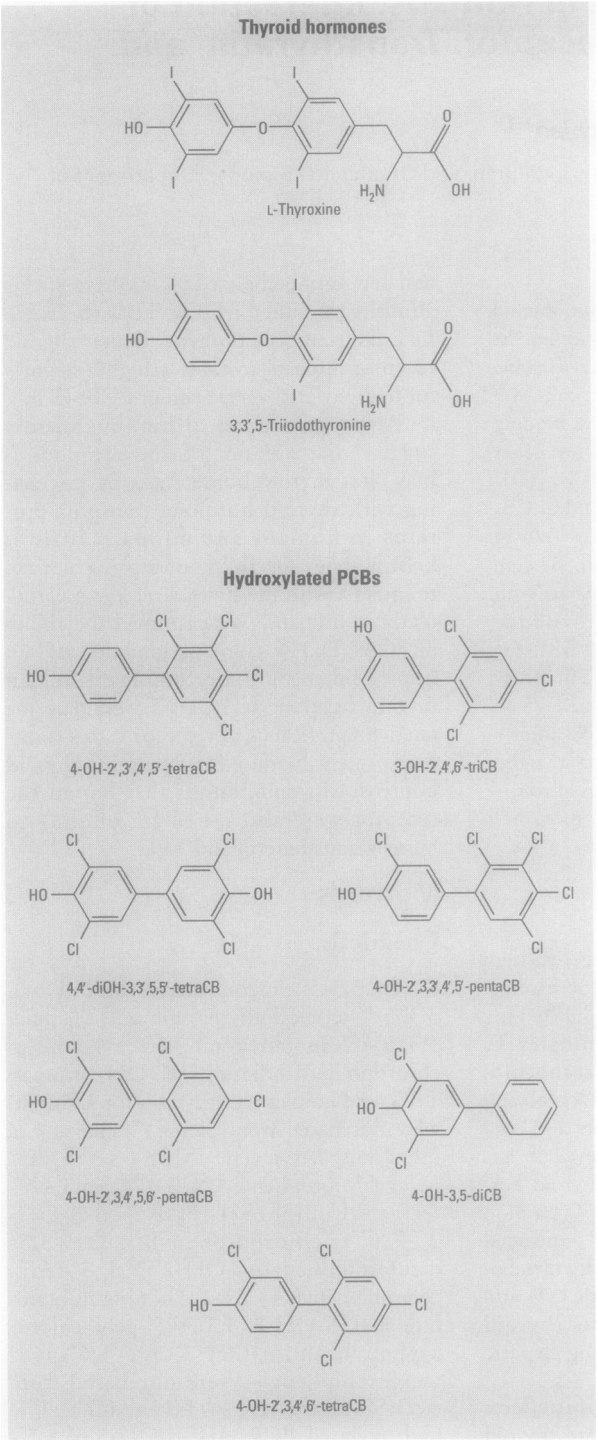

Figure 1. Structures of thyroid hormones and hydroxylated polychlorinated biphenyls (PCBs). CB, chlorinated biphenyl.

Ultra Scientific (North Kingstown, RI). The DDTs—1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2,2-trichloroethane (*o,p'*-DDT), 1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2-dichloroethane (*o,p'*-DDD), 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane (*p,p'*-DDT), and 2,2-bis(4-chlorophenyl)-1,1-dichloroethane (*p,p'*-DDE)—were purchased from Aldrich Chemical (Milwaukee, WI). All chemicals were dissolved in DMSO. DMSO did not exceed 0.01% in the binding assays.

Transfection

Sf9 insect cells (pupal ovarian cells from the fall armyworm) were purchased from Invitrogen (Carlsbad, CA) and cultured at 27°C in complete Grace's media (10% fetal bovine serum lactalbumin hydrolysate, tissue culture yeastolate, and glutamine; Invitrogen). A baculovirus phagemid containing the human thyroid receptor β1 cDNA was cloned into the multiple cloning site of the baculovirus phagemid pFastBac1 (Gibco BRL, Grand Island, NY) by DNA Technologies (Gaithersburg, MD). Confluent cells in a T-25 flask (Corning, Corning, NY) were rinsed with serum-free Grace's media and transfected with 1 µg phagemid DNA and 2 µl lipofectin in 2 ml serum-free Grace's media at 27°C. At the end of 5 hr, the transfection medium was replaced with complete Grace's media. After 7 days, medium containing baculovirus was harvested and stored at 4°C. To produce recombinant hTRβ, confluent Sf9 cells in a T-150 flask were incubated with 7 ml baculovirus-containing medium at 27°C for 1.5 hr. An additional 13 ml of Grace's media was added and cells were cultured for 5 days. Virus-containing medium was harvested and cell extracts were prepared.

Preparation of Protein Extract

Protocols for preparing cell extracts were modified from Toscano (*25*), Bres and Eales (*26*), and Sullivan et al. (*27*). Transfected Sf9 cells were scraped from the flask and centrifuged at 5,000 rpm for 5 min. The supernatant was harvested and stored at 4°C. The cell pellet was resuspended in 2.5 ml buffer A [10 mM Tris-HCl (pH 7.6), 10% glycerol, 3 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM dithiothreitol (DTT), 1 mM Pefabloc, 1 µg/ml aprotinin, and 20 µM leupeptin], incubated for 20 min on ice, and homogenized in a glass dounce. KCl was added to a concentration of 0.4 M and the homogenate was incubated on ice for 30 min, with shearing through a pasteur pipet every 10 min. The homogenate was then centrifuged at 25,000 rpm for 15 min at 4° C. The supernatant (cell extract) was aliquotted and stored at -80°C until use.

Immunodetection of TR Protein

Cell extracts were heated (95°C) in sodium dodecyl sulfate (SDS) loading buffer and electrophoresed through 10% SDS-PAGE. Gels were electroblotted onto polyvinylidene difluoride membranes (Sigma) at 25 V overnight. Membranes were rinsed in Tris-buffered saline (TBS; 10 mM Tris-HCl, pH 7.6, and 150 mM NaCl) and blocked in TBS supplemented with 3% bovine serum albumin, fraction V (Sigma). Membranes were incubated with a polyclonal antiserum to amino acids 62–82 of human TRβ1 (1:1250; Affinity BioReagents, Golden, CO) in TBST (TBS + 0.1% Tween-20) for 2 hr, rinsed 3 times in TBST, incubated for 1 hr with HRP-conjugated goat antirabbit IgG (Kirkegaard–Perry, Gaithersburg, MD), rinsed 4 times with TBST, and visualized with enhanced chemiluminescence (Amersham, Arlington Heights, IL).

TR Binding Assays

Saturation analysis. Cell extract containing hTRβ was added to assay buffer [10 mM Tris-HCl (pH 7.4), 10% glycerol, 5 mM DTT, and 0.5% CHAPS] to achieve a final concentration of 50 µg protein/ml in 100 µl total volume. Extracts were preincubated with 1,000-fold excess unlabeled T_3 for 15 min at 21°C, then increasing concentrations of [^{125}I]T_3 (0.05, 0.1, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 5, and 10 nM) were added and extracts were incubated for an additional 60 min. The reaction was terminated on ice and unbound [^{125}I]T_3 was separated from bound [^{125}I]T_3 with the addition of hydroxylapatite (HAP) slurry (1:1 v/v in 0.1 M KCl, 10 mM Tris-HCl, pH 7.4). Extracts were incubated in HAP slurry for 40 min, with vortexing every 10 min. The slurry was centrifuged at 5,000 rpm for 3 min and buffer was aspirated. HAP pellets were then washed 3–4 times with 400 µl wash buffer [0.1 M KCl, 10 mM Tris-HCl (pH 7.4), 0.5% CHAPS], resuspended in 200 µl slurry buffer, and counted in ScintiVerse scintillation fluid (Fisher, Houston, TX).

Competitive inhibition experiments. Based on the saturation analysis, 2.5 nM [^{125}I]T_3 was used in competitive inhibition experiments. Nonsaturable binding was estimated by preincubating cell extracts with 10,000-fold molar excess unlabeled T_3 for 15 min. Varying concentrations (10^{-8}–10^{-4} M) of unlabeled competitors were also

preincubated with cell extract before addition of [^{125}I]T_3. All other conditions were as described above.

To characterize the specificity of the recombinant receptor, the known TR agonists T_3, T_4, Triac, and Tetrac, and the inactive T_3 metabolite, rT_3, were tested in competitive inhibition experiments. We selected organochlorine compounds for testing based on their reported ability to alter *in vivo* responses or to bind serum transport proteins. PCB mixtures and some specific PCBs decrease serum T_4 levels, but PCB 126 and PCB 77 markedly enhance spatial learning in rats exposed *in utero*, while only slightly altering serum T_4 (*2*). Because the enhancement of learning is similar to that in hyperthyroid rat pups, Schantz et al. (*2*) proposed that PCBs 126 and 77 might be thyroid receptor agonists. We examined the interaction of these two compounds with the human thyroid receptor. Because several OH-PCBs bind TTR as effectively as T_4 (*22*), we also tested the ability of OH-PCBs to bind the receptor. DDTs and chloroacetanilides alter serum T_4 levels and catabolism, but their ability to bind TR is unknown.

Thyroid-binding Protein and Transthyretin Binding Assays

Conditions for these binding assays were modified from Lans et al. (*22*). Briefly, purified TBG or TTR was added to 200 µl assay buffer (TR assay buffer discussed previously) for a final concentration of 30 nM. We used 55 nM L-T_4 containing 100,000 cpm [^{125}I]L-T_4 to estimate total binding. Nonsaturable binding was estimated by preincubating protein solutions with 100-fold molar excess unlabeled L-T_4 for 15 min. Varying concentrations of unlabeled competitors were also pre-incubated with the protein before the addition of [^{125}I]L-T_4. All other conditions were as described for TR saturation analysis, except the volumes of HAP slurry and of slurry buffer for resuspension were doubled to account for the twofold larger assay volume.

Results

Immunodetection

Infected Sf9 cells expressed a protein of approximately 51 kDa that cross-reacted with a polyclonal antibody specific to hTRβ1 (Fig. 2). This protein is similar in size to the 52–55 kDa proteins recombinantly expressed in *Escherichia coli* (*28,29*).

TR Binding

Saturation analysis. Preliminary experiments using 50, 100, 200, and 300 µg protein/ml indicated that minimal nonsaturable binding (≤10% of total binding) occurred at 50 µg protein/ml. Similarly, varying incubation times (15, 30, 45, 60, 90, and 120 min) showed that a 60-min incubation period was sufficient for achieving equilibrium binding. Binding to recombinant hTRβ was saturable at 3 nM [^{125}I]T_3 with a K_d of 1.37 ± 0.24 nM (n = 4 experiments) and a B_{max} of 0.30 ± 0.09 nM (Fig. 3). The binding affinity observed with recombinant hTRβ expressed in Sf9 cells is similar to that reported for other recombinant thyroid receptors (*28,29*), but is lower than that reported for thyroid receptors extracted from tissues (approximately 0.1 nM) (*30*).

Competitive binding experiments. Thyroid hormones and their analog showed the expected order of affinity for recombinant hTRβ: Triac > T_3 = L-T_4 >> DL-T_4 ≈ Tetrac (Fig. 4 and Table 1). Of the xenobiotics tested, only the hydroxylated PCBs could inhibit 50% of [^{125}I]T_3 binding to hTRβ (Fig. 5 and Table 1), although with 10,000-fold lower potency than L-T_4. The coplanar PCBs, PCB 77 and PCB 126, did not displace T_3 from the receptor, nor did *p,p′*-DDE, *p,p′*-DDT, acetochlor, or methoprene. Although some displacement (20%) was achieved by the highest concentrations (100 µM) of *o,p′*-DDD, *o,p′*-DDT, DDOH, and alachlor, none of these compounds could inhibit 50% of [^{125}I]T_3 binding.

TTR and TBG Binding

The binding affinity of unlabeled L-T_4 was 62 ± 12 nM for TTR and 76 ± 15 nM for TBG. These affinities are in agreement with those reported by Lans et al. (*22*): 88–138 nM for TTR and 52–85 nM for TBG. All

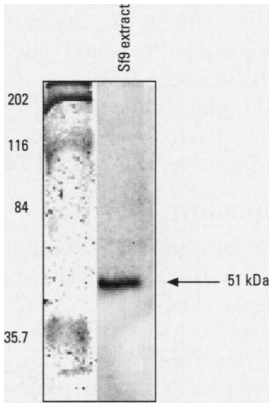

Figure 2. Western blot of cell extract from Sf9 cells infected with baculovirus containing the human thyroid receptor (hTRβ) gene. A 51-kDa protein that cross-reacts specifically with a polyclonal antibody to hTRβ1 is produced by the infected Sf9 cells.

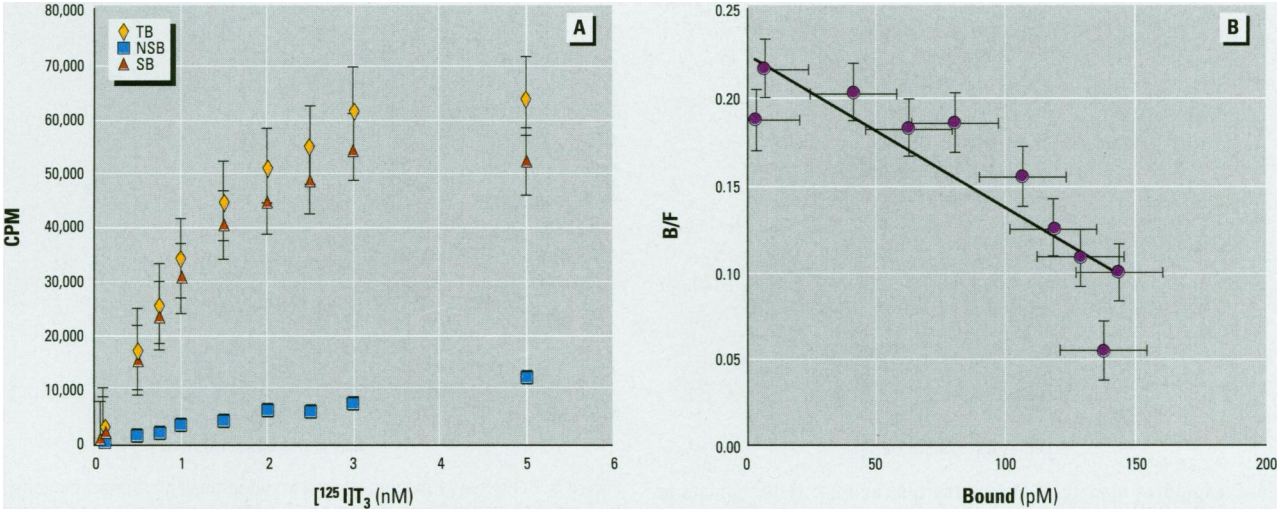

Figure 3. (A) Saturation analysis and (B) Scatchard analysis of [^{125}I]3,3′,5-triiodothyronine (T_3) binding to recombinant human thyroid receptor (hTRβ). At each concentration of [^{125}I]T_3, a 1,000-fold molar excess of unlabeled T_3 was used to estimate nonsaturable binding (NSB). Curves are the average ± standard error of four experiments. Abbreviations: TB, total binding; SB, saturable binding; B/F, bound/free concentration.

of the 4-hydroxylated PCBs bound TTR with affinities (10–140 nM) similar to that of the natural ligand L-T_4 (62 nM) (Fig. 6 and Table 1). Hydroxylation in the *meta* position appeared to abolish TTR binding (Table 1). PCB 126 bound TTR weakly, with a 1,000-fold lower affinity than L-T_4 and the 4-hydroxylated PCBs (Table 1 and Fig. 6). DDOH was the only DDT metabolite with weak affinity for TTR (approximately 100 µM).

Few of the xenobiotics competitively bound TBG. Two of the hydroxylated PCBs competed for TBG binding—4-OH-2′,3,4′,6′-tetraCB and 3-OH-2′,4′,6′-triCB (Fig. 7 and Table 1)—but with affinities 30–100-fold lower than L-T_4. Cl^- ions in the 2′,4′, and 6′ positions appeared to facilitate binding to TBG. Addition of an extra Cl^- at the 5 position seemed to abolish TBG binding, as 4-OH-2′,3,4′,5,6′-pentaCB did not compete for binding, whereas 4-OH-2′,3,4′,6′-tetraCB did. None of the other hydroxylated PCBs bound TBG. *o,p′*-DDD and DDOH bound TBG, but with affinities 70–800-fold lower than L-T_4 (Fig. 7 and Table 1). Acetochlor, alachlor, and methoprene showed no affinity for TTR or TBG.

Discussion

Of the four groups of compounds examined, only the hydroxylated PCBs bound to human TRβ1 with affinities ranging from 30–90 µM—affinities 10,000-fold lower than the natural ligand T_3. The hydroxylated PCBs had 1,000-fold greater affinities for TTR than for TR, making them competitors for the natural ligand L-T_4. Half of the hydroxylated PCBs tested had higher affinities for TTR than did T_4. Only two of the hydroxylated PCBs bound TBG—3-OH-2′,4′,6′-triCB and 4-OH-2′,3,4′,6′-tetraCB. In fact, 3-OH-2′,4′,6′-triCB did not bind TTR, but had a 20-fold

Table 1. Inhibition constants (± standard error, $n = 3$ experiments in duplicate) for thyroid hormones and environmental chemicals interacting with recombinant hTRβ, hTTR, and hTBG

	K_i (µM)		
Compound	TRβ	TTR	TBG
T_3	0.020 ± 0.015	–	–
L-T_4	0.024 ± 0.012	0.062 ± 0.012	0.076 ± 0.015
DL-T_4	0.135 ± 0.054	–	–
Triac	0.006 ± 0.005	–	–
Tetrac	0.075 ± 0.014	–	–
rT_3	0.808 ± 0.151	–	–
3-OH-2′,4′,6′-trichlorobiphenyl	90.3 ± 23.7	>100	5.46
4-OH-3,5-dichlorobiphenyl	83.5 ± 18.7	0.016	>100
4-OH-2′,3′,4′,5′-tetrachlorobiphenyl	37.7 ± 26.0	0.089 ± 0.009	>100
4-OH-2′,3,4′,6′-tetrachlorobiphenyl	43.0 ± 16.9	0.033 ± 0.002	2.34 ± 0.029
4-OH-2′,3,3′,4′,5′-pentachlorobiphenyl	67.2 ± 17.1	0.141	>100
4-OH-2′,3,4′,5,6′-pentachlorobiphenyl	36.5 ± 6.65	0.040 ± 0.008	>100
4,4′-diOH-3,3′,5,5′-tetrachlorobiphenyl	32.7 ± 12.1	0.011 ± 0.002	>100
PCB 77	>100	>100	>100
PCB 126	>100	140 ± 18.7	>100
o,p′-DDT	>100	>100	>100
o,p′-DDD	>100	>100	4.99 ± 4.13
DDOH	>100	89 ± 11	62.20 ± 40.9
p,p′-DDE	>100	>100	>100
p,p′-DDT	>100	>100	>100
Acetochlor	>100	>100	>100
Alachlor	>100	>100	>100
Methoprene	>100	>100	>100

Abbreviations: TRβ, thyroid receptor β; TTR, transthyretin; TBG, thyroid-binding globulin; T_3, 3,3′,5-triiodothyronine; T_4, thyroxine; Triac, 3,3′,5-triiodothyroacetic acid; Tetrac, 3,3′,5,5′-tetraiodothyroacetic acid; rT_3, 3,3′,5′-triiodo-L-thyronine; PCB 77, 3,3′4,4′-tetrachlorobiphenyl; PCB 126, 3,3′,4,4′,5-pentachlorobiphenyl; *o,p*-DDT, 1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2,2-trichloroethane; *o,p′*-DDD, 1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2-dichloroethane; DDOH, 2,2-bis(*p*-chlorophenyl)-ethanol; *p,p′*-DDE, 2,2-bis(4-chlorophenyl)-1,1-dichloroethane; *p,p′*-DDT, 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane.

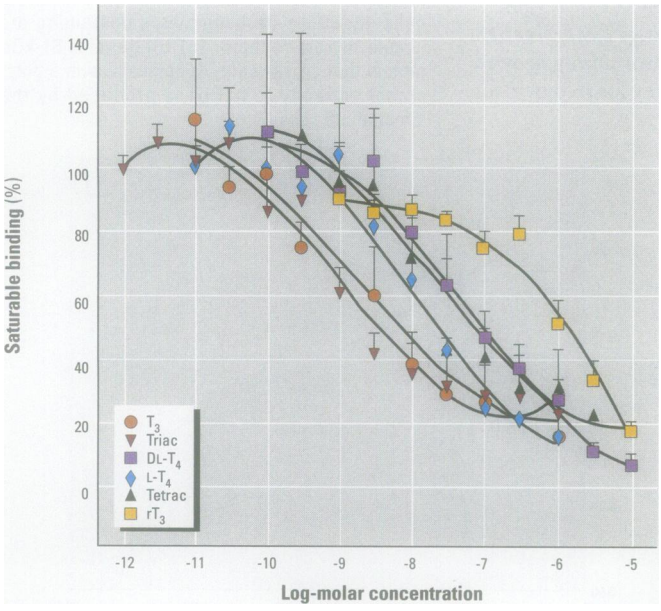

Figure 4. Competitive binding of known thyroid receptor (TR) agonists to recombinant human thyroid receptor β. Increasing concentrations of agonist competed with 2.5-nM [^{125}I]3,3′,5-triiodothyronine (T_3). Nonsaturable binding was estimated by incubation with 10,000-fold molar excess unlabeled T_3. $n = 3$ experiments performed in duplicate, except $n = 5$ experiments for T_3.

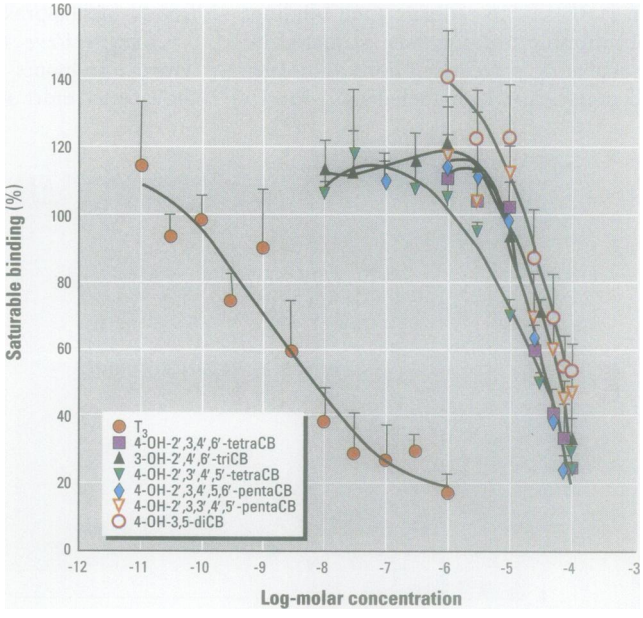

Figure 5. Competitive binding of hydroxylated polychlorinated biphenyls with recombinant human thyroid receptor β. Assay conditions are as described in Figure 4. $n = 3$ experiments performed in duplicate. Abbreviations: T_3, 3,3′,5-triiodothyronine; CB, chlorinated biphenyl.

greater affinity for TBG than for TR. PCBs and especially their hydroxylated metabolites interact with multiple components of the thyroid system, enhancing hepatic metabolism of thyroid hormones (*5,13–15*), competing for transport via serum proteins (*21*), especially TTR (*3,8,22–24*), and competing for receptor binding (this study). The physiological result is alteration in serum thyroid hormone levels. Of the known mechanisms, interaction with the thyroid receptor is likely to be less important than competition for serum transport proteins and induction of hepatic metabolism of T_4. Given K_i values of 30–90 µM for xenobiotics that interacted with the receptor, high concentrations on the order of 20 ppm (micrograms per gram) would have to be achieved in target tissues for hydroxylated PCBs to significantly alter T_3 binding to the receptor. Alternatively, the K_i values for TTR suggest that a concentration of only 0.017 ppm would have to be achieved for hydroxylated PCBs to significantly alter thyroid hormone transport via TTR. Hydroxylated PCB concentrations on the order of 0.36 ppm have been measured in human serum (*31*). Based on these data, *in vivo* disruption of TTR binding is more likely than disruption of receptor binding.

Neither of the coplanar dioxinlike PCBs, PCB 77 and PCB 126, bound the TR. Schantz et al. (*2*) observed that these PCBs accelerated spatial learning in rat pups exposed *in utero* and during lactation, an effect observed in hyperthyroid neonatal rats. Because both coplanar PCBs caused slight decreases in serum T_3 and T_4 but accelerated spatial learning, Schantz et al. (*2*) suggested that these PCBs might directly activate the thyroid receptor. In this study, PCB 77 and PCB 126 did not bind the human TRβ1, so direct interaction with the receptor probably does not explain the thyromimetic learning effect observed in the rats.

Previous work by McKinney et al. (*23*) indicated that two other coplanar PCBs, PCB 169 and PCB 80, bound to a rat nuclear extract with 100-fold lower affinities than L-T_4, whereas PCB 54, an *ortho*-substituted congener, did not bind at all. Although the present study did not examine the binding affinity of PCBs 169 and 80, reported differences in affinity for coplanar PCBs may be due to several factors. First, the current study used a protein extract of insect cells producing recombinant human TRβ1. Therefore, only a single TR isoform was available to interact with compounds in competitive binding experiments. In contrast, the rat liver nuclear extract probably contained not only rat TRβ1, but also TRα1 and TRα2 (*30*). Second, because only a single TR isoform was present in recombinant cell extracts, only TR homodimers could form, while in rat liver nuclear extracts, retinoid X receptors (RXRs), the heterodimeric partners of TR, were probably also present (*30,32*). *In vitro*, TR-RXR heterodimers exhibit different affinities for ligands than do TR-TR homodimers, although both appear to form spontaneously in cells (*30*). Third, species-specific differences in TR affinity for coplanar PCBs may exist.

DDTs and chloroacetanilide herbicides cause hypothyroidlike effects in animals, decreasing serum T_4 (*16,17,19–21*). None of these compounds bound to the thyroid receptor, indicating that they are unlikely to disrupt the thyroid axis via receptor interaction. DDOH bound to TTR and to TBG, but with such low affinity that concentrations in serum are unlikely to be high enough to compete for T_4 binding. *o,p′*-DDD bound to TBG with a fairly low affinity (5 µM) and is unlikely to reach such high concentrations in serum because of environmental exposure. However, clinical treatment of adrenal carcinomas resulted in 100–600 µM doses of *o,p′*-DDD (*20*). One consequence of *o,p′*-DDD treatment was decreased serum T_4, purportedly due to direct competition with *o,p′*-DDD for TBG binding (*20*). Our results support that hypothesis. Neither of the chloroacetanilides acetochlor nor alachlor bound TTR or TBG, but they enhance hepatic metabolism of T_4 in rats (*16,17*). Alteration of metabolism is probably the major mechanism by which chloroacetanilides affect thyroid axis function.

Our results suggest that disruption of thyroid hormone transport is one of the

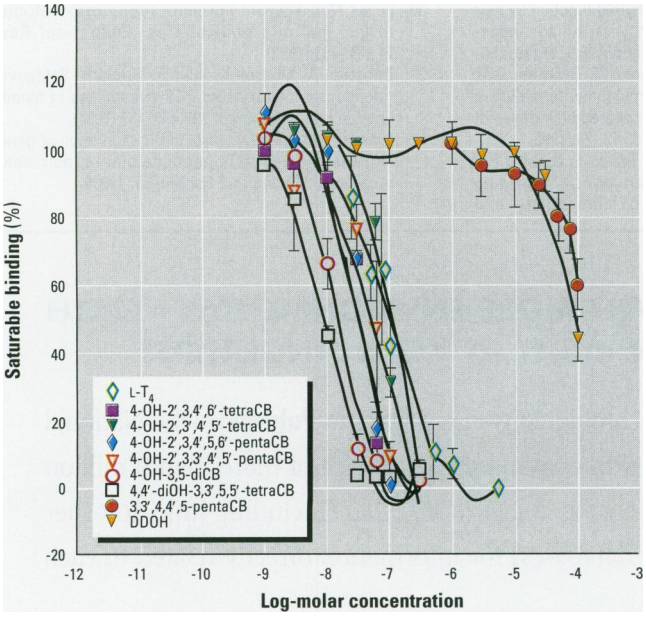

Figure 6. Competitive binding of OH-polychlorinated biphenyls (PCBs) with purified human transthyretin (TTR). Increasing concentrations of OH-PCBs competed with 55 nM L-thyroxine (T_4) spiked with 100,000 cpm [^{125}I]L-T_4. Nonsaturable binding was estimated by incubation with 100-fold molar excess unlabeled L-T_4. n = 3 experiments performed in duplicate. Abbreviations: DDOH, 2,2-bis(*p*-chlorophenyl)-ethanol; CB, chlorinated biphenyl.

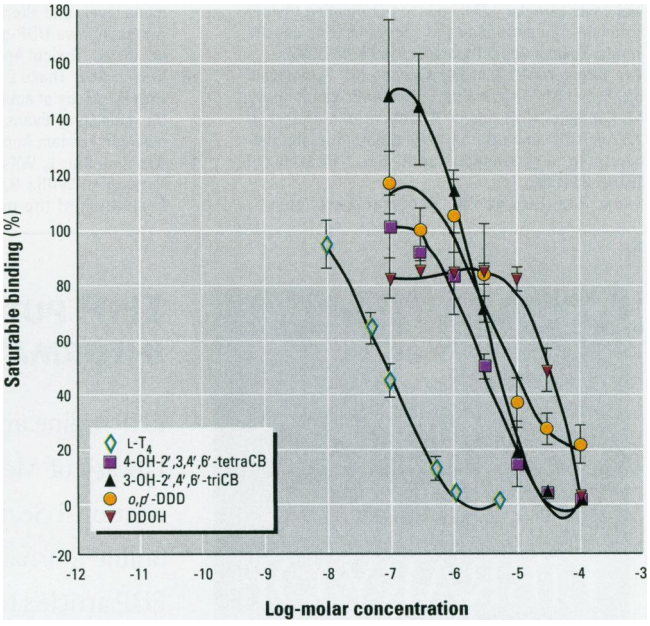

Figure 7. Competitive binding of hydroxylated polychlorinated biphenyls with purified human thyroid-binding globulin. Assay conditions are as described in Figure 6. n = 3 experiments performed in duplicate. Abbreviations: T_4, thyroxine; CB, chlorinated biphenyl; *o,p′*-DDD, 1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2-dichloroethane; DDOH, 2,2-bis(*p*-chlorophenyl)-ethanol.

mechanisms by which organochlorine compounds alter thyroid homeostasis. In particular, hydroxylated PCBs compete effectively for T_4 binding to TTR, but few compounds compete for TBG binding, even at μM concentrations. TBG is found only in some mammals, including primates, ungulates (cattle, sheep, goats, pigs, water buffalo, and horses), and carnivores (dog), but not in rodents (rat) or lagomorphs (rabbit). Depending on species, TBG binds 60–90% of serum T_4. Interestingly, TBG deficiency in humans does not interfere with euthyroid status, suggesting that TTR is also important for T_4 transport in humans (*1*). TTR is a highly conserved TH binding protein in all vertebrate species (*1*), so disruption of thyroid hormone transport by hydroxylated PCBs could potentially occur in all vertebrates, not only in humans.

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